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URINARY EXCRETION OF VANILMANDELIC ACID AFTER $+G_x$ IMPACT IN HUMANS

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FOREWORD

This study was conducted during January 1965 by the Biodynamics Branch of the 6571st Aeromedical Research Laboratory.

The author wishes to acknowledge the cooperation and assistance of all members of Dynalectron Corporation, Land-Air Division, Aeromed Sub-Group.

* * *

This technical report has been reviewed and is approved.

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ABSTRACT

Seven volunteer subjects were exposed once each to 25 $+G_x$ impact and sham impact on the Daisy Decelerator. Urinary excretion of vanilmandelic acid (VMA) was measured during two timed periods prior to and after impact or sham impact. The results indicate that the average urinary excretion of VMA increases with exposure to both impact or sham impact. The greatest average increase was observed after true impact. It is suggested that subject anxiety attendant to both experimental conditions causes an increased liberation of catecholamines. True impact may further stimulate this adrenergic activity.

I

INTRODUCTION

Exposure of human subjects to G_x impact (abrupt deceleration) commonly produces a transient episode of hyper-reflexia, skeletal muscle tremor and general hyperactivity. These effects appear to be related to the level of impact force and are especially noticeable above 20 G_x. Rhein and Taylor (Ref. 1) postulated that these responses could be due to subject pre-run anxiety with accompanying sympathicotonia. They also suggest that impact might produce a transient alteration in peripheral or central nervous system function which subsequently results in hyper-reflexia. However, quantitative data to support these hypotheses have not been established.

The present investigation is an evaluation of sympatho-adrenal activity in human subjects before and after experimental impact runs. We have studied the urinary excretion of vanilmandelic acid (VMA) in seven male subjects prior to and after exposure to 25 $+G_x$ on the Daisy Decelerator (Ref. 2). Secretly scheduled sham runs for each subject served as an experimental control.

II

METHODS

A. Experimental Design

Seven healthy young men who were volunteer subjects on active duty in the Air Force were selected to participate in this study. The physical characteristics of these men are summarized in Table I. All of the subjects had been exposed to impact acceleration studies for over a year and were familiar with the Daisy Decelerator and restraint systems utilized. Each subject was contacted separately and informed that he would participate, over a four week period, in two 25 $+G_x$ impact runs and two sham runs. The order of the runs would not be known and the project was not to be discussed with other sled riders.

Only two runs were actually conducted with each subject; one impact and one sham in a sequence established by dice shake as shown in Table I. Investigative personnel assigned to the project and technicians who operated the Daisy Decelerator were also instructed not to disclose the sequence of the impact/sham runs.

Table I. SUBJECT CHARACTERISTICS AND ORDER OF RUNS

Initials	Age	Ht(cm)	Wt(kg)	Order of Runs: Impact	Order of Runs: Sham
G.W.C.	21	174	79	I	II
J.O.E.	26	184	84	II	I
C.W.H.	23	184	74	I	II
J.L.P.	22	181	79	I	II
C.A.R.	23	180	73	II	I
J.W.R.	23	182	75	I	II
T.R.T.	25	184	71	I	II

Four timed urine specimens were collected as follows: from 3 hours to 1 hour pre-impact (B-1); from 1 hour to 5 minutes pre-impact (B-2); from 5 minutes pre-impact to 1 hour post-impact (R-1) and from 1 hour post-impact to 3 hours post-impact (R-2).

True impact runs were programmed for 25 $\pm$ 1 G applied in the $+G_x$ vector, sled entrance velocity at water brake 10 m/second with an onset of deceleration of 1000 G/second. Sham runs were conducted exactly as a true run except the Daisy air piston, which propells the sled, was partially depressurized 10 seconds prior to the firing time. As a result, the sled moved down the track but failed to contact the water brake.

B. Subject Protocol

Two experiments were conducted per day, one in the morning and one in the afternoon. Subjects were not permitted to observe the run preceding their own. Throughout the experimental period subjects

were instructed to avoid certain foods containing vanillin, which would ultimately interfere with the chemical analysis for VMA. For morning runs the subjects ate no breakfast while afternoon subjects ate no lunch. Water consumption, ad lib. was encouraged to maintain adequate urine output.

On the day of a run the subject reported to the Daisy Decelerator facility approximately one hour before the firing time. The B_1 urine specimen was collected. The subject then received a pre-run physical examination and was instrumented for vectorcardiogram, blood pressure (phonic transducer sphygmomanometer, Airsearch Mfg. Co.) and respiratory rate (nasal thermistor, Yellow Springs Inc.). Physiological information was received on a Sanborn 350 recorder via instrumentation cable. Approximately 10 minutes prior to the scheduled firing time the subject walked to the Daisy Decelerator sled and was restrained with a standard harness consisting of shoulder straps, lap belt, foot straps and D-ring grips for hand holds. A second pre-run urine specimen (B-2) was collected just prior to strap-in. Count-down and firing of the sled usually occurred within 5 minutes after strap-in.

Immediately after impact the restraint harness was released and the subject examined for evidence of injury. A post-run physical examination followed 10 minutes after the impact. Personnel receiving sham runs were questioned as to how well the experiment was concealed.

The first post-impact urine specimen (R-1) was collected at the Daisy Decelerator area while the remaining post-impact specimen (R-2) was collected by the subject at a specified time and returned to the laboratory.

C. Handling of Specimens

The volume of urine specimens were measured to the nearest milliliter. If the measured volume was equal to less than 3.0 ml per minute excretion rate the specimen was diluted with distilled water to the equivalent rate. Triplicate 50 ml aliquots of the urine were acidified with 0.1 ml of 12 N HCl and stored in screw-capped bottles at -20°C to await analysis.

D. Chemical Analysis

Urine specimens were analysed for VMA using the method of Pisano et. al. (Ref. 3), in which VMA is first extracted from urine with ethyl acetate and subsequently re-extracted into aqueous $K_2 CO_3$.

VMA is then oxidized with $NaIO_4$ to produce vanillin which is measured spectrophotometrically (347 mμ) against VMA standards receiving the same treatment.

III

RESULTS

The results of these experiments are presented graphically in Figure 1. These data are expressed as the average urinary excretion of VMA (μg/min) for all subjects during two timed intervals before and after impact or sham exposure. Standard error of the mean is represented by brackets ($\pm$ 1 $S\bar{x}$).

Urine specimen B-1 collected one hour prior to the scheduled experiment is quite similar for both impact (4.4 $\pm$ 1.6 μg/min) and sham (4.2 $\pm$ 1.3 μg/min). These values are also in close agreement with baseline VMA excretion[1] reported for healthy young men prior to participation in thermal stress experiments (Ref. 4). VMA excretion increases in period B-2 and R-1 for both impact and sham experiments. In period R-2, VMA excretion continued to increase after impact while it diminishes slightly after sham. The relative changes in VMA excretion are illustrated in Figure 2, which expresses the quantitative differences between the average impact and sham excretion rates. This treatment of the data seeks to identify an increment of VMA excretion due to impact exposure. It appears that increased VMA excretion in the R-2 urine specimen may represent some effect of impact.

Statistical analyses of these experimental data are summarized in Table II. Experimental differences were evaluated by comparing the B-1 specimen with each of the subsequent specimens (B-2, R-1 and R-2). For intra-experimental analysis the impact specimens were compared with the corresponding sham specimens. Both parametric (Ref. and non-parametric (Ref. 6) methods were used. It is apparent from these analyses that the changes observed within and between the impact and sham experiments are of marginal statistical significance. This is partially explained by the unusual variability in several subjects combined with the small number of subjects.

[1] 4.3 $\pm$ 0.5 μg/min

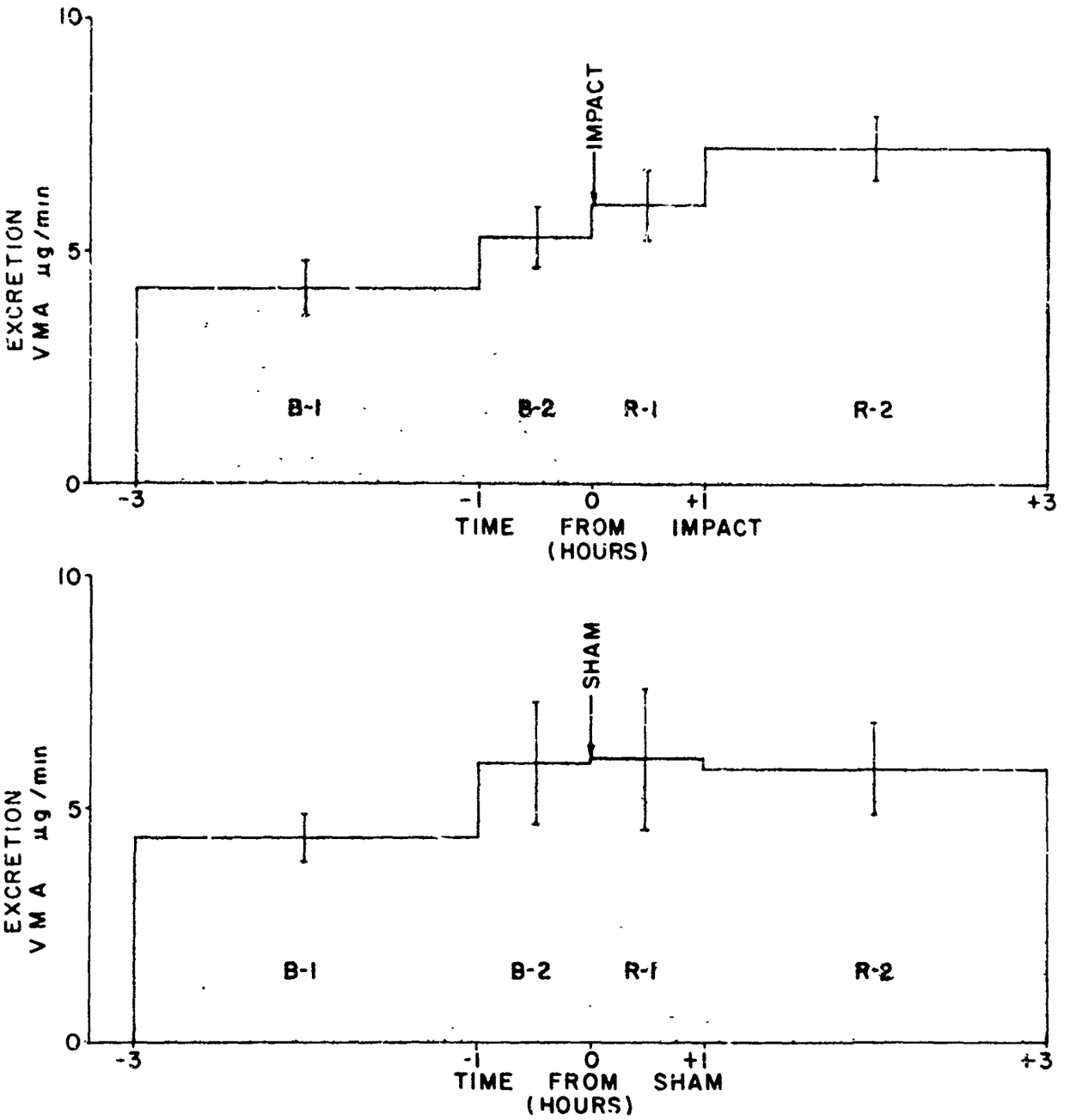

Figure 1. Urinary Excretion of VMA Prior to and Following Impact and Sham Acceleration

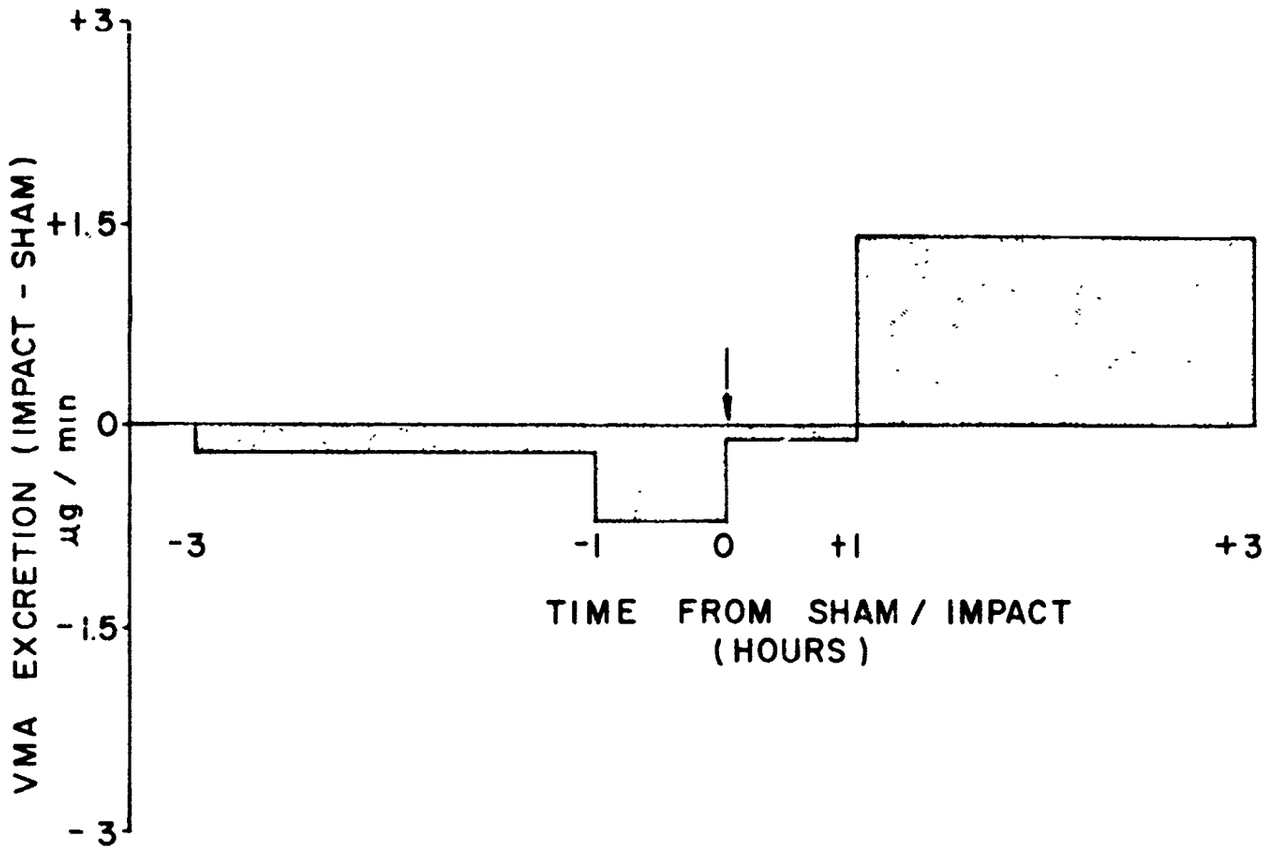

Figure 2. Relative Excretion of VMA Due to Impact

IV

DISCUSSION

The metabolic fate of catecholamine secreted from either the adrenal medulla or sympathetic nervous aystem includes O-methylation to form metanephrine or normetanephrine which are secreted either unchanged or subsequent to conjugation with sulfuric or glucuronic acid. Deamination of metanephrine and normetanephrine yields vanilmandelic acid[2] (VMA) which is the predominant excretion product of catecholamine origin (Ref. 7). A small percentage of free epinephrine and norepinephrine are excreted in the urine of humans.

[2] 3-methoxy-4-hydroxy mandelic acid

TABLE II. SUMMARY OF INTER- AND INTRA-EXPERIMENTAL STATISTICAL COMPARISONS

Groups Compared[1]	t-value[2]	P	T-value[3]	P
B-1 (+) vs B-2 (+)	1.25	≤ 0.25	-21	--
B-1 (+) vs R-1 (+)	1.97	≤ 0.10	3	≤ 0.10
B-1 (+) vs R-2 (+)	1.72	≤ 0.20	4	≤ 0.20
B-1 (-) vs R-2 (-)	1.13	≤ 0.30	-5	--
B-1 (-) vs R-1 (-)	1.06	≤ 0.40	-10	--
B-1 (-) vs R-2 (-)	1.36	≤ 0.20	-5.5	--
B-1 (+) vs B-1 (-)	0.26	≤ 0.90	11	--
B-2 (+) vs B-2 (-)	0.48	≤ 0.70	11	--
R-1 (+) vs R-1 (-)	0.06	≤ 0.90	9	--
R-2 (+) vs R-2 (-)	0.71	≤ 0.50	-11	--

1. Impact experiment (+), sham experiment (-)
2. Students' 't": $t_{.05}$, df 7 = 2.36
3. Wilcoxons' "T": $T_{.05}$, df 7 = $|2|$

The measurement of VMA, therefore, provides a rough index of endogenous catecholamine secretion although the original source of the VMA molecule is not determined (Ref. 8).

Studies by Goodall and Berman (Ref. 9) using centrifugation and mock centrifugation as stressors provide a partial basis for comparison with our studies. Their investigations demonstrated that epinephrine excretion increased significantly prior to and during centrifugation and mock centrifugation and attained similar levels during both treatments. Ninety minutes after exposure to either real or mock centrifugation epinephrine excretion diminished markedly. Urinary excretion of norepinephrine increased significantly only after true centrifugation. Increases in either epinephrine or norepinephrine were accompanied by a concomitant increase in VMA excretion. These measurements suggest that epinephrine excretion is related to the anticipation of centrifugation while norepinephrine excretion responds to gravitational stress which produces hemodynamic alterations mediated through the sympathetic nervous system.

In the present experiments subject urinary VMA excretion increased prior to impact and sham exposure. This observation is consistent with the hypothesis of subject pre-exposure anxiety accompanied with sympatho-adrenal hyperfunction. Post-impact and post-sham VMA excretion remains elevated, which indicates that sympatho-adrenal hyperfunction continues well after the time of maximum anxiety. In addition, the greater average excretion of VMA in specimen R-2 following true impact indirectly suggests that impact force may produce an additional transient augmentation in catecholamine secretion. Hyper-reflexia, increased reactivity of skeletal muscle and subject hyperactivity which commonly are present immediately after impact exposure may be manifestations of a sudden increase in catecholamine release attendant to impact exposure (Ref. 10). This hypothesis, of course, lacks rigorous statistical support in these data.

A comprehensive study of catecholamine excretion under similar conditions with a larger subject population will be required to establish which portion of the sympatho-adrenal system is possibly actuated by impact force.

REFERENCES

1. Rhein, L.W. and E.R. Taylor: Increased Skeletal Muscle Activity Following Impact. ARL-TDR-62-26, Dec. 1962.

2. Chandler, R.F. The Daisy Decelerator. 6571st Aeromedical Research Laboratory, Holloman AFB, New Mexico (In press).

3. Pisano, J.J., J.R. Crout and D. Abraham: "Determination of Urinary 3-Methoxy-4-Hydroxy Mandelic Acid." Clin. Chem Acta 7: 289, 1962.

4. Hanson, P.G., R.E. Johnson and G. Engle: "Plasma Free Fatty Acid Changes in Man During Acute Cold Exposure and Nicotinic Acid Ingestion." Aerospace Med. 36: No. 11, 1054, 1965.

5. Snedecor, G.W. Statistical Methods. Ames Iowa, Iowa State College Press, p 87, 1956

6. Siegel, S. Nonparametric Statistics. New York: McGraw-Hill Co., p. 75, 1956

7. Armstrong, J.D., A. McMillan and K.N. Shaw: "3-Methoxy-4-Hydroxy-D-Mandelic Acid, Urinary Metabolite of Norepinephrine." Biochim et Biophys Acta. 25: 422, 1957.

8. Wurtman, R.J.: "Catecholamines," New Engl. J. Med., 273, No. 14, 746, 1965.

9. Goodall, McC. and M.L. Berman: "Urinary Output of Adrenaline, Noradrenaline, and 3-Methoxy-4-Hydroxy Mandelic Acid Following Centrifugation and Anticipation of Centrifugation." J. Clin. Inv. 39, 1533, 1960.

10. Rothballer, A.B. "The Effects of Catecholamines on the Central Nervous System." In: Symposium on Catecholamines, Baltimore, Williams and Wilkins Co., 494, 1959.

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